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SEMI-AUTOMATED X-Y-PLOTTER-BASED METHOD FOR MEASURING ROOT LENGTHS¹

W. W. WILHELM, J. M. NORMAN, AND R. L. NEWELL²

Abstract

Root-length measurements are an important aspect of many agronomic research programs. Several automated systems have been reported which use modifications of the line-intersect technique to estimate root length; however, most of these systems are very costly. This paper describes an inexpensive automated system using a modified line-intersect technique to estimate root length. An X-Y plotter was used to move a light sensor in a grid pattern through the projected photographic image of a root system, recording intersections with an event counter. Compared to manual counting using the line-intersect technique, the system described accurately estimated root length of samples up to 10 m in length ($r^2 = 0.98$). The advantage of our system is that costs are reduced by using equipment that is readily available at most laboratories: X-Y plotter and slide projector. The cost of additional items was less than \$600. Each determination of root length required 50 sec. The accuracy of the system was comparable to others that are based on the line-intersect technique.

Additional index words: Newman technique, Line-intersect method.

ROOT-LENGTH measurements are required for agronomic investigations varying from descriptive work on depth and distribution of roots to work involving the measurement of water and nutrient uptake. All aspects of root studies are costly in terms of capital, labor, and time.

The technique for estimating root length described by Newman (1966) has been used by several researchers (Rowse and Phillips, 1974; Arkin et al., 1977³; Richards et al., 1979; Voorhees et al., 1980). All of these techniques use a modification of the Newman technique suggested by Marsh (1971) and tested by Tennant (1975). The Newman technique is based on the premise that length of a root system (any set of curved lines) is directly proportional to the number of intersections (N) between the root system and a random direction straight line and surface area (A) of the container confining the root system, and inversely proportional to the total length of straight

lines (H) used to determine the intersections:

$$\text{Root length} = \frac{\pi NA}{2H} \quad [1]$$

The modification suggested by Marsh (1971) and tested by Rowse and Phillips (1974) and Tennant (1975) indicated that, if the random direction straight lines are replaced with a set of parallel lines distance D apart, the area (A) in Eq. [1] would be represented by HD. Substituting into Eq. [1]

$$\text{Root length} = \frac{\pi ND}{2} \quad [2]$$

They also suggested that, if D was set equal to $2/\pi$, the number of intersections would be a direct measure of root length in units of D. If two perpendicular sets of parallel lines are used (a grid system), Eq. [2] would become

$$\text{Root length} = \frac{\pi ND}{4}$$

and D would set equal to $4/\pi$.

Several researchers have reported the development of apparatus to automate the Newman (1966 line-intersect method of estimating length of root systems (Rowse and Phillips, 1974; Richards et al., 1979) and projected images of root systems (Arkin et al., 1977³; Voorhees et al., 1980). These authors, as well as Tennant (1975) and Reicosky et al. (1970), reported data indicating that similar degrees of accuracy could be achieved with automated modifications of the line-intersect technique as with manual measurements, with considerable time savings with the automated systems.

This report describes an X-Y-plotter-based, semiautomated system for measuring root length from projected images of washed root systems that is considerably less expensive than those described in previous reports.

Materials and Methods

System Description

The root-length measuring system (Fig. 1) has six major components: (1) slide projector, (2) projection stand, (3) X-Y plotter and controller (computer or microprocessor), (4) light sensor and amplifier, (5) power supply, and (6) event counter. A standard Kodak⁴ 850H slide projector was used to project the pho-

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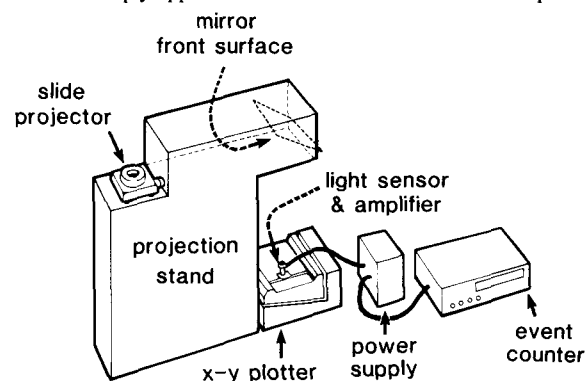


Fig. 1. Schematic of major components of the root-length measuring apparatus.

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³ Arkin, G. F., R. Chaison, and W. R. Jordan. 1977. Using your desktop calculator to estimate root length. *Agron. Abstr.* p. 79.

tographed image of root systems mounted in slide holders onto the plotter surface. Roots were suspended in their storage solution (1:1, ethanol:water) and photographed in a manner similar to that described by Voorhees et al., (1980), using a 457 by 356 by 25 mm acrylic plastic pan to contain the roots. With back-lighting and Kodak Panatomic-X film (FX-135) developed as a negative, the roots appeared white on a black background. The projection stand (constructed of plywood) provided a fixed focal length between the projector and the plotter platen. A front-surface mirror was positioned at a 45° angle to reflect the root image onto the plotter platen with no distortion or multiple images as found with normal back-surface mirrors. A 100-mm scale was photographed with each root system so the image could be projected to its original size; however, by measuring the size of the scale, the degree of magnification could be determined if magnification of the root system is desired. The plotter (Tektronix 4662) was used as a device to move the light sensor-amplifier assembly in a 10- by 10-mm grid pattern. The Motorola MFOD102F fiber optic PIN photodiode (0.2-mm-diam aperture) and amplifier were mounted on the pen carriage. The photoreceptor of the photodiode pointed up, toward the mirror (image). A schematic of the light sensor-amplifier assembly is shown in Fig. 2. The number of intersections between the root system and grid pattern was displayed on a Simpson 7026 frequency counter used in the event counting mode. The threshold on the event counter was set as high as possible without getting count on a blank slide (photographed with no roots in the acrylic plastic pan). It was essential to locate the amplifier very near the photodiode to minimize noise problems associated with the extremely low current arising from the photodiode. In addition, the wires supplying power and connecting the amplifier output to the counter must be very flexible and well shielded if the pulses arising from real intersections are to be repeatable.

Calibration Procedure

The system accuracy was determined in several ways. First, known lengths (0.05 to 10.00 m) of sewing thread (0.24-mm diam), fine wire (0.14-mm diam), and carpet thread (0.60-mm diam) were photographed and projected on the plotter platen. Manual and electronic counts were made to compare the count-

ing efficiency of the automated system. The projected image was always focused on the plane in which the light sensor traveled to insure sharp images. Secondly, various combinations of sewing thread, wire, and carpet thread were photographed and counted manually and electronically to simulate the effect of various-sized roots on counting efficiency. Thirdly, random corn (*Zea mays* L.) root samples of various sizes were selected, photographed, and counted manually and electronically. The guidelines for manual counting defined by Tennant (1975) were followed.

Roots that were stained were treated with one drop of a 1% solution of crystal violet dissolved in ethanol per 10 ml of storage solution and allowed to stand in the staining solution for 30 min after gentle mixing. After being rinsed twice with ethanol and once with distilled water, roots were stored for a limited time (< 2 weeks) in water at 2 C.

Calibration curves were fitted to data points using nonlinear regression.

Results and Discussion

Calibration of the X-Y-plotter-based, automated system, using measured lengths of thread and wire, indicated the system could accurately measure length from the number of intersections if the calibration curve was of a quadratic form (Table 1). Examination of the data pre-

Table 1. Quadratic equation coefficients ($Y = B_0x + B_1x^2$), coefficients of determination (r^2), and standard error (SE) for comparison of electronically and manually counted intersections from known lengths (0.05 to 10.00 m) of sewing thread, carpet thread, and fine wire using a 10-mm grid.

Calibration material	Coefficient			SE
	B_0	B_1	r^2	
Sewing thread	1.002	-0.0001	0.99	3.72
Carpet thread	0.997	-0.0001	0.99	2.26
Fine wire	0.996	-0.0001	0.99	3.13
Combination	0.998	-0.0001	0.99	3.02

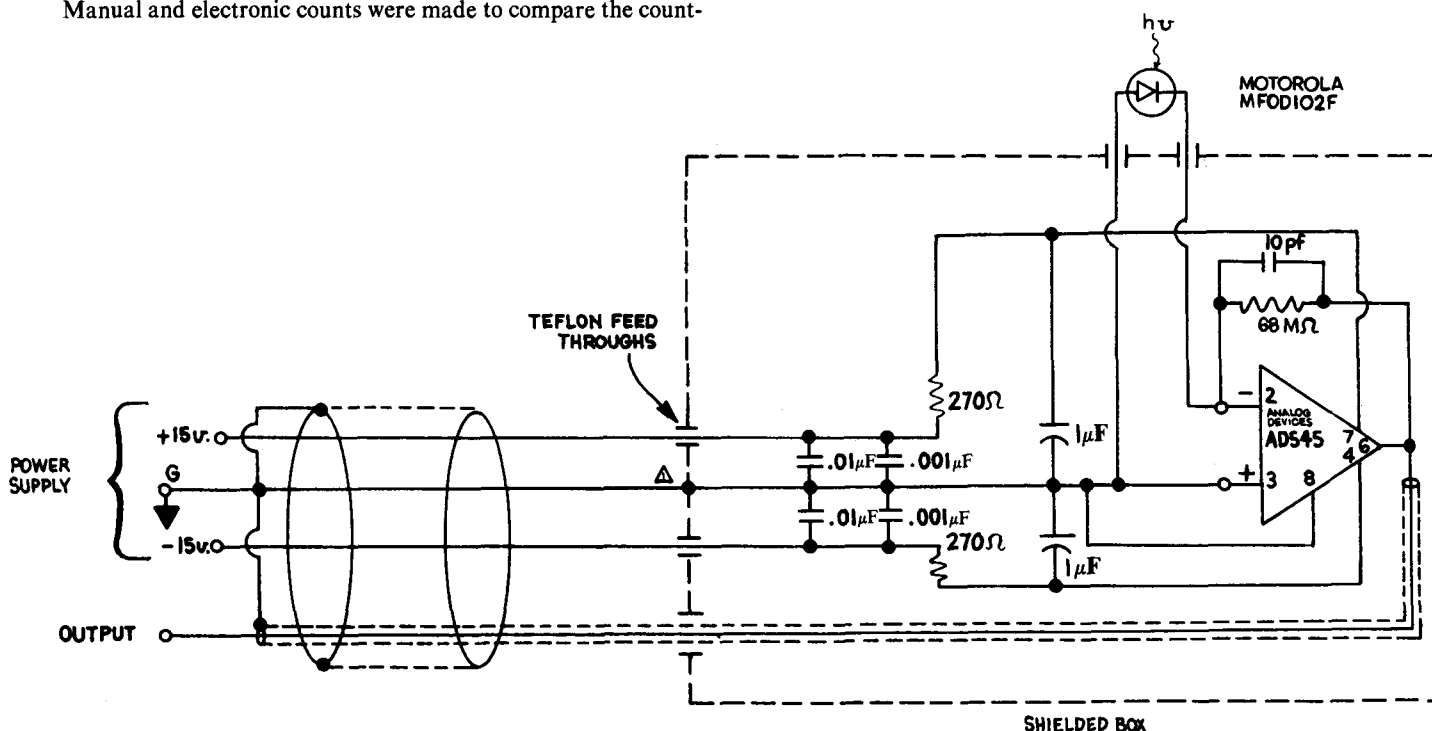


Fig. 2. Schematic of electronic circuitry for root length digitizer preamplifier. The 0.01-, 0.001-, and 1.0-μF capacitors, which are noise-suppression components, are grounded at point Δ.

sented in Fig. 3 indicated that, when attempts were made to measure lengths greater than 6 m, the number of intersections between grid lines and calibration material detected by the system did not remain linear. This was a result of insufficient separation of material and too much overlapping of calibration material segments. When the

Table 2. Quadratic equation coefficients ($Y = B_0x + B_1x^2$), coefficients of determination (r^2), and standard error (SE) for comparison of intersections determined electronically and manually between various-sized root samples and 10-mm grid. Number of intersections ranged up to 1,200 with each of the sources of root samples.

Root sample	Coefficient			SE
	B_0	B_1	r^2	
Greenhouse, unstained	0.325	-0.0001	0.97	35.87
Field samples, unstained	0.424	0.0	0.83	81.69
Field samples, stained	1.095	-0.0004	0.98	40.68

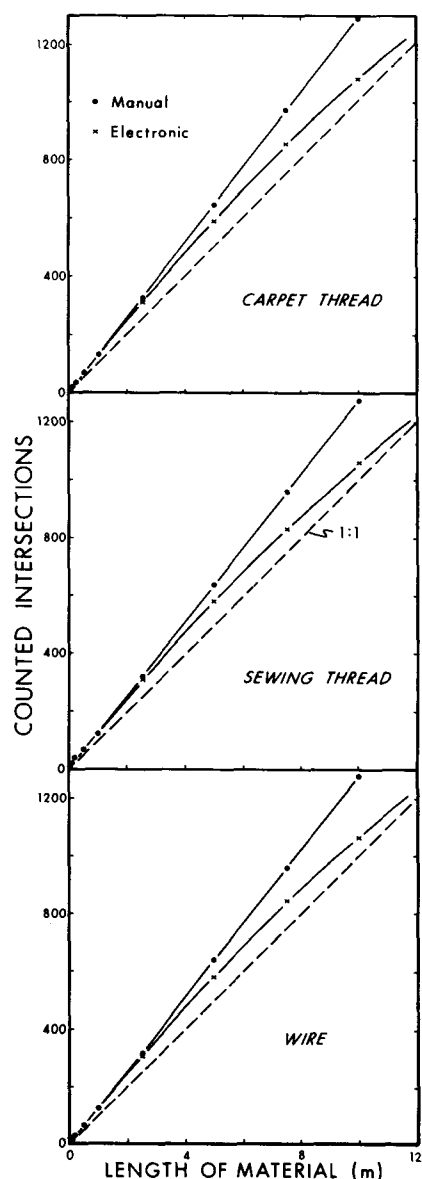


Fig. 3. Relationship between length of calibration material and manual and electronic counts for various lengths (0.05 to 10.00 m) of thread and wire.

quadratic equation was used and forced through the origin, r^2 values all exceeded 0.99 (Table 1). Use of combinations of various lengths of string, thread, and wire also gave highly accurate estimates of the measured length ($r^2 > 0.99$). In addition, intersections were counted manually and electronically using 0.050- and 0.089-mm-diam wire. Correlations between these counts were greater than 0.99 in all cases. This indicated that roots as small as 0.050 mm in diameter can be detected by the counting system with no magnification.

Calibration of the system with root samples on which length was estimated with manual counts using the same grid system as produced by the plotter and employing the equation defined by Marsh (1971) to convert intersections to root length was not as successful as using thread and wire. The r^2 values were somewhat lower than desired (Table 2) with unstained greenhouse and field samples. Coefficients of x in the calibration equation indicated underestimation of intersections. Much greater contrast between background and small roots was achieved with stained roots. The B_0 coefficient became similar to that found for thread and wire (Table 1 and 2), and r^2 increased to 0.98. Similar results were found if Kodak ortho film (6556, Type 3) was used instead of stain to obtain higher contrast. The choice of film and staining is largely up to the researcher and may be limited by the research objectives. The authors prefer Panatomic-X film and staining because of the greater exposure range allowed by this film. In cases where specific research objectives preclude the use of stains, Kodak ortho film would be preferred.

Time is critical in all root-length measuring techniques. Scanning one image required approximately 50 sec. One scan per sample was sufficient to achieve the degree of accuracy reported here. The changing of samples (slides) was very rapid, requiring the operator to advance the projector (focusing was automatic with the projector described), reset the event counter, and initiate the plotting routine by pushing a button on the plotter.

The system rapidly and accurately determined the number of intersections between photographic images of thread, wire, and stained root samples, using equipment and technology readily available at almost every research location. The cost of our system was minimal because we relied on the more complete use of equipment already available (X-Y plotter and controller and slide projector). The cost of the other items was approximately \$600 (November 1981 prices). Even if the X-Y plotter and slide projector we used were included, the cost was less than \$5,500. With a dedicated board-level microprocessor and inexpensive X-Y plotter, an entire stand-alone system could be fabricated for less than \$2,000.

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